PLANT HOST AFFILIATION AND REDESCRIPTION OF *PHYTOMYZA SUBTENELLA* FROST (DIPTERA: AGROMYZIDAE)

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Abstract.—The biology of *Phytomyza subtenella* Frost (Diptera: Agromyzidae) was observed for the first time. This species was found to be a seed feeder on the hemiparasitic Indian paintbrush, *Castilleja miniata* Douglas (Orobanchaceae), in Colorado. Biological observations are provided, along with a redescription of the species and illustrations of the male genitalia. Additionally, the 17 orobanch-feeding agromyzids are discussed, along with comments on several possible lineages, based on similarities of male genitalia, within the 13 orobanch-feeding *Phytomyza.*

Key Words: Agromyzidae, *Phytomyza subtenella,* Orobanchaceae, *Castilleja miniata,* host plant

The agromyzid genus *Phytomyza* Fallén contains more than 450 described species, all of which have internally feeding, plant-parasitic larvae (Spencer and Steyskal 1986). Most species of *Phytomyza* are leaf-miners, but some are known to feed in other plant parts, including stems, roots, flowers, and seeds. Members of this genus attack species in nearly 30 plant families, although most individual *Phytomyza* species are highly restricted in diet, usually monophagous or feeding on few closely related plant species (Spencer 1990, Scheffer and Wiegmann 2000). Within *Phytomyza,* morphologically similar species tend to feed on closely related plants (those in the same genus or family) in a pattern suggestive of host-associated radiations (e.g., Scheffer and Wiegmann 2000). This type of pattern has been discussed for a number of other

groups of highly specialized insects (Ehrilch and Raven 1964, Becerra 1997, Farrell 1998, Becerra and Venable 1999), although the processes giving rise to such patterns are not fully understood. A critical first step in investigating either the patterns or processes involved with insect diversification is gaining a clear understanding of the host affiliations of the species of interest.

Agromyzid species attacking leaves generally form an externally visible tunnel or "leafmine" as they feed, making them conspicuous to entomologists. In contrast, those species that feed deeply within tissues such as stems, roots, flowers, or fruits often provide no external symptom of their presence. These species are not readily discovered without targeted collection followed by dissection of plants. Not surprisingly, our knowledge of such species and their
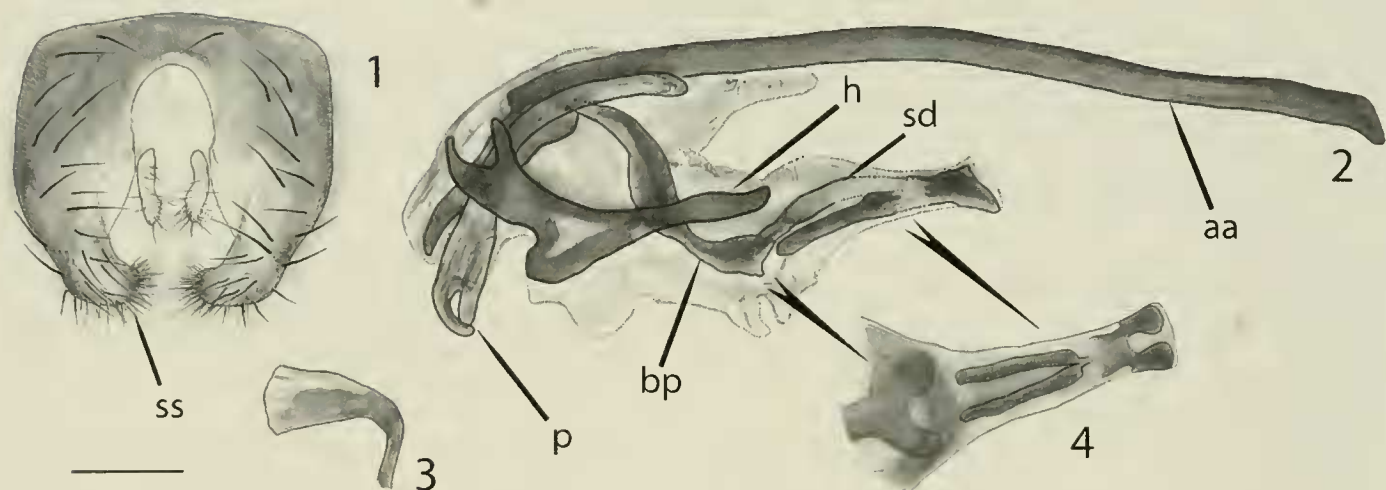
Figs. 1–4. *Phytomyza subtenella,* ♂ genitalia; scale bar = 0.1 mm; aa = aedeagal apodeme, bp = basiphallus, h = hypandrium, p = paramere, sd = sperm duct, ss = surstylus. 1, Epandrial complex, dorsal view. 2, Aedeagal complex, lateral view. 3, Ejaculatory apodeme, lateral view. 4, Distiphallus, ventral view.

host affiliations is fragmentary, limiting our ability to fully investigate host-use evolution within Agromyzidae.

Although most *Phytomyza* species are leafminers, a number are known to feed in stems or seeds (Spencer 1990), including one group of primarily seed feeders that has apparently radiated onto plants in the Orobanchaceae. Currently, in North America and Europe, there are 13 *Phytomyza* species known from this plant family (Table 1). Although phylogenetic relationships among these and other *Phytomyza* species have not been explored, genitalic similarities suggest that there are several distinct lineages within this group, with unknown affinities among these lineages.

Spencer (1969) first alluded to the possibility that *Phytomyza subtenella* Frost is a seed (or stem) feeder, citing the elongated form of the ovipositor, and later commenting that it is "almost certainly an internal feeder" (Spencer 1981). Here, we report the host plant for this species, having reared it from a hemiparasitic Indian paintbrush, *Castilleja miniata* Douglas (Orobanchaceae). We also report observations on its natural history and provide a redescription and more detailed drawings of genitalia than were previously available in the literature (only the basiphallus had been figured thus far, cf. Spencer and Steyskal 1986: figs. 1148, 1149).

METHODS AND RESULTS

Morphological terminology follows McAlpine (1981) and Spencer (1987).

Voucher specimens of the flies have been labeled as such and deposited in the California State Collection of Arthropods (CSCA) in Sacramento, CA, and the National Museum of Natural History (USNM) in Washington, DC; voucher specimens of the plants (LSA#1, 3, 5) have been deposited in the Herbarium of the Rocky Mountain Biological Laboratory, Gothic, CO.

In late summer 1996, numerous fly puparia were observed in fruits of *C. miniata* (voucher specimen LSA #3) at Emerald Lake, Colorado. Pupae were collected from these fruits in September and were stored in 1 oz. plastic portion cups, half at room temperature and half in a 0°C freezer in an attempt to simulate overwintering conditions. Adult emergence by either method was low (<5% of ~200 puparia), possibly due to the change in microclimate by removal from the field. In July of the following year, adult flies were collected from the bracts of *C. miniata.*

TAXONOMY

Phytomyza subtenella Frost
(Figs. 1–4)

Phytomyza subtenella Frost 1924: 89. Type locality: "Mount Rainier (Washington)".

Type: LT♀ in USNM (designated by Frick 1959, by assumption of holotype). Subsequent references: Frick 1952: 428 (catalog); Frick 1959: 436 (inadvertent lectotype designation, diagnosis, illustrations of head and wing); Frick 1965: 805 (catalog); Spencer 1969: 26 (geography), 221 (key), 276 (diagnosis, distribution, illustration of basiphallus); Spencer 1981: 365 (key), 425 (redescription, distribution, illustration of basiphallus, comment on relationship with *Phytomyza lupini* Sehgal); Spencer and Steyskal 1986: 186 (key, diagnosis, distribution, illustration of basiphallus, comment on relationship with *Phytomyza eumorpha* Frey).

Diagnosis.—Face and frons mostly yellow; gena high; face and gena strongly receding in lateral view; epistoma absent; 4 fronto-orbital setae, posterior 2 reclinate, anterior 2 convergent. Notum and scutellum silver pruinose; dorsocentral setae 1+3; single row (2–6 pairs) of acrostichal setulae; forecoxa yellow; pleuron silvery-grey pruinose, except yellow only along dorsal and posterior borders of anepisternum, dorsal edge of meron, and surrounding posterior spiracle; femora dark silver pruinose, yellow apically; tibiae and tarsi dark. Abdominal tergites and sternites brown, with yellow posterior edges. In male, paramere bilobed distally, with inner lobe straight, and outer lobe curving and overlapping with inner; basiphallus gently S-curved in lateral view with edges along middle of curve widened slightly, strongly bilobed distally in ventral aspect; apex of distiphallus a single, large, membrane-bounded opening with sclerotization giving appearance of being bilobed. In female, oviscape 1.3–1.6× longer than basal width, shining brown, with ring of long setae distally.

Adults, ♂, ♀.—Body length 2.3–2.8 mm. *Head:* Mostly yellow, except ocellar region and behind head (median occipital sclerite, occiput, and postgena) silvery-grey pruinose, clypeus and palpus light brown, prementum and lateral part of antennal scape

brown, and first flagellomere and arista black. Postocellar setae strong, divergent. Ocellar setae proclinate, as strong as posterior orbital setae, with bases same distance apart as posterior ocelli. Frons 1.5–2.0× wider than long, and 2.0–2.8× wider than eye width (when viewed from dorsal aspect); with 4 fronto-orbital setae, posterior 2 reclinate and subequal, anterior 2 convergent and subequal or with anteriormost one smaller; with row of small proclinate setulae between fronto-orbital setae and orbital margin, anteriorly becoming slightly larger and convergent. Lunule 0.09–0.12 mm high. Gena high, 0.6–0.7× higher than eye height. Eye 1.1–1.2× higher than wide (when viewed from lateral aspect). One strong oral vibrissa, with several smaller setulae along oral margin. Face and gena distinctly receding in lateral view. *Thorax:* Notum and scutellum entirely silvery-grey pruinose. Chaetotaxy: 1+3 dorsocentral setae (occasionally with fine supernumerary setae), with cluster of setulae just anterior to anteriormost seta; single row (2–6 pairs) of acrostichal setulae; 1 presutural intra-alar seta; 1 postsutural intra-alar seta; 1 postalar seta; 1 postpronotal seta (with several supernumerary setulae); 2 notopleural setae; 2 pairs scutellar setae; otherwise bare, with few supernumerary setulae. Pleuron silvery-grey pruinose, except yellow along dorsal and posterior borders of anepisternum, dorsal edge of meron, and surrounding posterior spiracle. Chaetotaxy: 1 proepisternal seta; 3–4 anepisternal setae along posterior edge, middle 1–2 strong, outer ones fine, occasionally with 1–2 fine setae in dorsal part, anterior of this edge; 2 katepisternal setae, posterior one stronger. *Legs:* Forecoxa yellow, getting darker in dorsal ⅓; mid- and hindcoxae dark. Femora silvery-grey pruinose, yellow distally. Tibiae and tarsi dark brown. *Wing:* Length 2.4–2.8 mm. Distance between end of R_1 and R_{2+3} 1.4–1.9× longer than that between R_{4+5} and M_{1+2}. Halter yellow. *Abdomen:* Tergites and sternites brown pruinose, but yellow along posterior edges. *Male genita-*

lia (Figs. 1–4): Epandrium rounded, with small setae in addition to covering of small hairs; surstylus fused to epandrium, with densely clustered hairs, many directed medially (Fig. 1). Cerci small. Hypandrium U-shaped from dorsal view, with medially directed process near middle, and with bilobed process distally on each posterior arm (Fig. 2). Paramere (postgonite of other authors) bilobed distally, with inner lobe straight, and outer lobe curving and overlapping with inner (Fig. 2). Ejaculatory apodeme 2.0× longer than high, with distal edge fan-like (Fig. 3). Aedeagus with basiphallus gently S-curved in lateral view, with edges along middle of curve widened slightly (Fig. 2); basiphallus in ventral aspect strongly bilobed distally (Fig. 4). Distiphallus sclerotized in three parts: central sperm duct (Fig. 2), paired, elongated, anteriorly-directed sclerites, and paired posteriorly-directed apical sclerites (Fig. 4); elongated sclerites confluent with apical sclerites through membrane; apex of distiphallus a single, large, membrane-bounded opening, despite appearance of being truly bilobed. *Female terminalia:* Oviscape 0.36–0.46 mm wide at base, length 1.3–1.6× greater than basal width; shining brown, with ring of long hairs distally; with light covering of silver-grey pruinosity in basal ¼.

Type material.—LT♀ (USNM), Mt. Rainier, Wash[ington]., above Longmire's, 5,000 ft., Aug. 3, [19]05 [date handwritten]/ Type No. 50021 U.S.N.M [red; number handwritten]/Phytomyza subtenella Frost [handwritten; black submargin].

Other material examined.—USA: Wisconsin, Polk Co., July, Baker (1PLT♀, USNM); California, El Dorado Co., Echo Lake [2,250 m el.], E.I. Schlinger, 23-VII-1955 (1 ♂, 1 ♀, USNM); Colorado, Gunnison Co., Emerald Lake, 3,150 m el., 39°00'41"N 107°02'32"W, reared from *Castilleja miniata*, 5.XI.1996 (emer. III-1997), Lynn Adler (1 ♂, 2 ♀, CSCA; 1 ♂, 1 ♀, USNM), hand collected from *Castilleja*

miniata, VII-1998, Lynn Adler (1 ♂, 3 ♀, CSCA; 1 ♂, 1 ♀, USNM).

Distribution.—Canada (Alberta, British Columbia); United States (California, Colorado, Washington, Wisconsin, Wyoming). The single paralectotype from Wisconsin is unambiguously labelled (Polk Co. Wis., July, Baker), although the presence of this species in northwestern Wisconsin (where the elevation is below 500 m), seems unlikely, as all other known specimens are from the Rocky Mountains and the Sierra Nevada, at elevations above 2,000 m. Spencer (1969) even comments that this species is likely to be limited to this high elevation western zone, despite the record in Wisconsin, which he also considered dubious.

Behavioral observations.—Adult *P. subtenella* were observed on leaves and bracts of *C. miniata* in late July 1998 at Emerald Lake, Colorado, where most were in flower. Pairs of adults, or occasionally three adults, were observed in what appeared to be mating positions.

Approximately two weeks later, adult flies were no longer observed, but fly larvae were common inside the locules of maturing fruits. Up to three larvae were found per single fruit. Because there was no obvious damage to these fruits (i.e., no evidence of larval entry holes), it seems likely that oviposition was directly into fruits. Larvae consumed seeds while leaving behind the netlike outer seed coat, causing extensive damage (Adler 2002).

By early September, *C. miniata* fruits had matured and begun dehiscing, and the flies had pupariated. These puparia were found both inside and outside fruits. In the latter case, exit holes were evident in the capsule wall and at times puparia were found protruding through these holes. The duration of the puparial stage in the field is not known; field collected puparia emerged after several months in the laboratory.

DISCUSSION

The group of *Phytomyza* presented in Table 1 all attack Orobanchaceae, parasitic

Table 1. *Phytomyza* species known to feed on plants in the family Orobanchaceae, arranged by host genus; * = new record; † = probable feeding site. All host genera are in the hemiparasitic tribe Rhinantheae, except for *Orobanche* which is in the holoparasitic tribe Orobancheae.

Species	Distribution	Host Genus	Feeding Site
P. subtenella Frost*	Nearctic	*Castilleja*	seeds
P. trivittata Frost	Nearctic	*Cordylanthus*	seeds†
P. rostrata Hering	Palaearctic	*Euphrasia, Melampyrum, Odontites, Rhinanthus*	stems
P. affinis Fallen	Palaearctic	*Euphrasia*	seeds
P. flavofemorata Strobl	Palaearctic	*Melampyrum*	seeds
P. isais Hering	Palaearctic	*Odontites*	seeds
P. orobanchia Kaltenbach	Palaearctic	*Orobanche*	seeds, stems
P. diversicornis Hendel	Palaearctic	*Pedicularis*	stems
P. pedicularicaulis Spencer	Nearctic	*Pedicularis*	stems
P. pedicularidis Spencer	Nearctic	*Pedicularis*	seeds
P. pedicularifolii Hering	Palaearctic	*Pedicularis*	leaves
P. tenella Meigen	Holarctic	*Pedicularis*	seeds
P. varipes Macquart	Holarctic	*Rhinanthus*	seeds

plants that obtain water and nutrients from other plants via root connections. Several species of this group have unknown affinities, but genitalic similarities indicate several distinct lineages. For example, one seed-feeding species, *P. flavofemorata* Strobl attacking *Melampyrum* L., appears, by genitalic similarity, to belong to a group with three leaf-feeding species attacking non-orobanchs, including *P digitalis* Hering attacking *Digitalis* L., and *P. crassiseta* Zetterstedt and *P. veronicicola* Hering attacking *Veronica* L. (Plantaginaceae, Digitalideae). The species *P. trivittata* Frost, attacking *Cordylanthus* Nutt. ex Benth., appears very closely related by nearly identical male genitalia to *P. chelonei* Spencer, a species attacking *Chelone* L. (Plantaginaceae, Cheloneae). There appear to be at least two other distinct groups based on genitalic similarities. The first includes two stem feeders (*P. diversicornis* Hendel and *P. pedicularicaulis* Spencer, both attacking *Pedicularis* L.) and two seed feeders (*P. affinis* Fallén and *P. tenella* Meigen, the former attacking *Euphrasia* L. and the latter *Pedicularis*). The second includes the seed feeder *P. subtenella* (attacking *Castilleja* Mutis ex L.f.) and the seed/stem feeding *P. orobanchia* Kaltenbach (attacking species

of the holoparasitic genus *Orobanche* L.), but also includes several additional species whose host affiliations remain undiscovered (e.g., *P. eumorpha* Frey and *P. orindensis* Spencer), or attack species of the non-orobanch genus *Lupinus* L. (Fabaceae) (*P. lupini* Sehgal). Interestingly, *P. orobanchia* of this latter group has been shown to be of potential importance in controlling *Orobanche* species parasitizing tomatoes in Ethiopia (Elzein et al. 1999).

Two *Phytomyza* species, *P. lupini* and *P. lupinivora* Sehgal, feed on hosts of the genus *Lupinus*, which is in a family quite distinct from Orobanchaceae. The latter species, known only from the female holotype, is a leafminer, while the former species is an internal stem or flower-head feeder, even pupariating internally. Interestingly, Spencer (1981) speculated on a relationship between *P. lupini* and *P. subtenella*, based on unstated similarities of the male genitalia, being distinguished by different states of the male epistoma above the mouth margin (which is lacking in *P. subtenella*) and the color of the forecoxa. Comparing genitalia, the aedeagus is very similar in shape and size from the lateral aspect, and the distiphallus is very similar from ventral aspect; the same holds true for the genitalia of *P.*

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